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# CHEMORECEPTION IN THE BLUE CRAB, *CALLINECTES SAPIDUS*

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Chemoreception plays a dominant role in the feeding behavior of marine crustaceans. A logical first step in evaluating the precise role that sapid chemicals play in food gathering is to measure the animal's sensitivity to different levels of such substances. In this work behavioral criteria are utilized to measure the sensitivity of the blue crab, *Callinectes sapidus*, to a solution derived from natural food.

Studies using behavior to determine crustacean sensitivity to various amino acids and other substances have been few and have included only two studies of crustaceans other than the lobsters, *Homarus americanus*, (McLeese, 1970, 1974), *H. gammarus* (Mackie and Shelton, 1972; Mackie, 1973) and *Panulirus argus* (Levandowsky and Hodgson, 1965). In one other study, Fuzessery and Childress (1975) have compared the chemosensitivity of five crustaceans, the mysid, *Gnathophausia ingens*, the galatheid crab, *Pleuroncodes planipes*, the anomuran crab, *Pagurus hirsutiusculus*, the carid shrimp, *Spirontocaris taylori*, and the brachyuran crab, *Cancer antennarius*. Hindley (1975) has studied the ability of the prawn, *Penaeus merguiensis*, to detect amino acids. Where comparisons of sapid substances have been made, food extracts have generally been seen to be more effective in releasing feeding activity than even fairly complex synthetic mixtures (McLeese, 1970; Mackie, 1973).

The aims of this study were to observe the feeding behavior of the blue crab under laboratory conditions and select those components of the feeding repertoire that appeared to be most sensitive to the presence of sapid substances, utilize the selected behavioral components to determine the threshold concentration at which a food extract is detected, and examine the effects of food deprivation upon the detection threshold.

## Materials and Methods

After preliminary observations of the normal feeding behavior of the blue crab, *Callinectes sapidus*, a chemosensory testing apparatus was constructed in order to observe the response of crabs to various levels of sapid solutions. After numerous trials with the apparatus, two experiments were carried out: the first to establish thresholds for the detection of sapid material and the release of feeding, and the second to study the effect of food deprivation upon the thresholds.

For the preliminary observations and the first experiment, blue crabs, *Callinectes sapidus*, collected with dipnets and traps from the Shrewsbury River and northern Barnegat Bay, New Jersey, were transported in separate aerated containers in order to minimize handling stress. Mating pairs were not separated during transport or laboratory holding. The water at the site of the crabs' capture was 20.9° C (±2.4 s.d.) and 21.7‰ (±1.5 s.d.) For the second experiment, an unexpected early winter necessitated trawls of less than ten minutes in the Navesink River and

Sandy Hook Bay in order to obtain crabs in sufficient number. The crabs were carefully sorted into separate containers for transport. Capture by trawling is not as desirable as capture by less stressful methods, but short trawls and careful net handling considerably reduce handling stress. The water in the Navesink River was 9.2° C and 20.6‰.

At Sandy Hook Laboratory, a maximum of 72 crabs was maintained under natural photoperiod in a rectangular tank (4.8 × 1.8 × 0.5 m) with a sand bottom. Salt water from Sandy Hook Bay was recirculated through a gravel-sand-oyster shell filter before percolation through the sand on the tank's bottom with new water continuously added to maintain water quality. Both mating pairs and molting individuals were isolated. An *ad libitum* diet of blue mussels, *Mytilus edulis,* was provided. Crabs began to feed usually within hours of capture. During the first experiment from July to September, 1976, the temperature and salinity of water in the tank were 21.2° C (±0.7 s.d.) and 23.6‰ (±2.4 s.d.), respectively. During the second experiment, from November 1976 to January 1977, the crabs were gradually brought to and held at 20.6° C (±1.2 s.d.) and 23.9‰ (±0.4 s.d.)

The chemosensory testing apparatus was designed to present the crab with a sapid solution and to observe the response. Individual crabs were tested while isolated in 3.25-liter polystyrene chambers covered with white translucent plexiglass. Sea water passed through wound cellulose filters and a heat exchanger before entering a header tank with a gravel-sand-oyster shell filter. Water siphoning from the header tank entered each chamber *via* two lengths of plastic tubing, one carrying the main flow and the other used for adding the experimental solution. Both flows mixed as they entered the chamber. Flow rates were adjusteed to between 0.6 to 1.0 liter/min. A blind with view ports surrounded the water table upon which 18 chambers were arranged in a staggered line. Trials with dye solutions showed that 20 ml of solution injected within five sec into the inflowing water would disperse rapidly and completely through each chamber. To determine a dilution factor for estimating effective concentrations within a chamber, solutions of methylene blue were injected and the optical densities of water samples from the downstream end of the chamber compared with those of standard dilutions of the dye solution in a spectrophotometer at 663 nm. The maximum concentration occurred in the chamber between 0.75 and 1.0 minute injection of the solution. The maximum dye concentration within the chamber was $5.25 \times 10^{-3}$ times the original concentration for a 1.07 liter/min flow rate and $4.85 \times 10^{-3}$ times the original concentration for 0.60 liter/min flow rate. Using these measurements, estimated effective chambers concentration of the experimental solutions throughout this study was calculated by multiplying the concentration of the injected solution by $5 \times 10^{-3}$.

The sapid solution presented to blue crabs was a seawater solution of freeze-dried extract of hard clam, *Mercenaria mercenaria.* Preparation as well as chemical analysis of the freeze-dried clam extract (FDCE) was performed by the Southeast Utilization Research Center of the National Marine Fisheries Service. The dry weight composition of the FDCE was 53.7% protein, 5.9% fat, 7.9% ash, and 32.7% undetermined. Most of the amino acids comprised between one and two per cent of the FDCE dry weight. For the most abundant amino acids the dry weight composition was 4.2% taurine, 4.1% glutamic acid, and 3.2% aspartic acid.

On the day preceding each four-day period of testing, a stock FDCE solution was prepared as follows: first, a quantity of FDCE was ground in a mortar until powdered; secondly, a weighed portion of the powdered FDCE was mixed with sea water that had been filtered through a 0.4 $\mu$m membrane, and the resultant solution stirred for two hours with a magnetic stirrer; thirdly, the solution was filtered through tared Whatman No. 4 and GF/C filter paper; and fourthly, the concentration of the stock FDCE solution was corrected for the loss of the filtrant, which averaged 28.8% ($\pm$2.3) of the initial FDCE weight.

After preparation, the stock FDCE solution was refrigerated, and dilutions were made with membrane-filtered sea water approximately one hour before each day's testing.

Observation of an individual crab commenced at least one minute prior to injecting a FDCE dilution. The crab's posture and activities, presence of feces and regurgitated shell, and the extent of gill bailing were noted. Then, with the observer blind to the identity of the dilution, the FDCE was injected and observations of the crab's behavior recorded at 0.5-minute intervals for three minutes. Behavior was scored on the basis of criteria that were developed after observation of normal feeding and numerous trials in both experimental and holding tanks. A sharp increase in the antennule flicking rate accompanied by abrupt onset of continuous and vigorous gill bailing, all occurring within 1.5 minutes after FDCE introduction and continuing for at least 1.0 minute after onset, indicated detection. Feeding was considered to begin with chelae probing.

In the first experiment crabs were placed into the chambers between 1100 and and 1200 and tested the following day between 0900 and 1100. For 15 days, 18 blue crabs per day were presented with five dilutions of a 1.5 g/liter FDCE solution and a control of membrane-filtered sea water. The order of presentation and choice of dilution were taken from a random number table, except that the crabs that were active were passed over until they became still. Individual crabs were not retested without at least a two-day residence in the holding tank. Molting and mating crabs were not tested.

In the second experiment for four days, 18 crabs per day were tested as in the first experiment except that the number of dilutions was expanded from five to eight in order to present lower FDCE concentrations. Then, to investigate the effect of food deprivation on the detection threshold, the crabs were held without food for six days and retested in the same manner as before.

Regression analysis (Draper and Smith, 1966) was used to estimate the detection threshold. The threshold concentration was taken to be the concentration at which 50% of the crabs responded and was calculated from the regression equation relating the percentage of crabs responding and the logarithm of the FDCE concentration.

## Results

### *Normal feeding behavior and the selection of response criteria*

Blue crabs would begin searching for food when juice from chopped clams, *Mercenaria mercenaria,* was dripped into an aquarium. A crab would rise from its resting or buried position and walk with its chelae extended and held just above

the sand surface. Progress would be halting because the dactyls would move in arcs over the sand and occasionally probe beneath its surface. If a dactyl or chela contacted a piece of clam, the clam was quickly scooped inward and forward by the dactyls or grabbed by one chela and brought to the mouth to be torn by the maxillipeds and ingested. In its search a crab would walk over and away from a clam portion if neither a dactyl nor chela happened to contact the clam.

Crabs on an *ad libitum* diet of mussels would probe the mussel pile with dactyls and chelae and then separate one mussel from the rest by cutting the byssus threads with a chela. The crab would crack the shell with a chela and pry open the valves with both chelae as one would open a book. The chelae would then bring the opened mussel or bits of it to the mouth and hold it as the maxillipeds scraped the tissue from the shell. Large pieces of shell not ingested would be spit out after being scraped clean. The whole sequence typically would take three to five minutes with most of the time being spent in separating one mussel from the others.

During preliminary observations in the testing apparatus after the introduction of the FDCE, a sequence of behaviors was observed, the extent of which depended on the FDCE concentration. At high FDCE concentrations ($10^{-2}$ to $10^{-4}$ g/liter), an increase in the rate of antennule flicking and the beginning of or increase to continuous gill bailing occurred and always preceded feeding and grooming behaviors. A rise in posture and the gaping and occasional labiating of the maxillipeds closely followed the antennule flicking and gill bailing. As antennule flicking and gill bailing continued, the crabs moved the dactyls in arcs touching the bottom. Such dactyl searching was preceded or accompanied by movements of the chelae probing toward the bottom. Crabs would attempt ingestion of shell bits or feces by bringing the material to the mouth with the chelae and then either spitting out the material or dropping it. Grooming usually followed feeding movements. The body was groomed by rubbing and picking motions of the dactyls and chelae, and the buccal area, by picking with the dactyls and chelae and rubbing with the palps. The palps also groomed the dactyls and chelae as well as the eyes, antennules, antennae and other mouthparts. Occasionally during grooming, an individual probed with the chelae or attempted ingestion, but usually grooming continued uninterrupted. Grooming entailed much rising and settling but little or no walking. At the highest FDCE level ($10^{-2}$ g/liter) an abrupt rise in posture, immediate defecation, and vigorous grooming sometimes followed FDCE presentation. Attempted ingestion after defecation was rare and occurred usually near the end of observation.

At intermediate FDCE concentrations ($10^{-4}$ to $10^{-8}$ g/liter) the full sequence was not observed. After walking and dactyl searching, the crab resumed a standing or resting position. Often instead of food search movements, grooming followed the initial increases in antennule flicking and gill bailing.

At low FDCE concentrations ($10^{-8}$ to $10^{-12}$ g/liter) only the increase in the rate of antennule flicking and the onset of continuous gill bailing occurred. Sometimes there was a rise in posture, but no subsequent feeding behavior followed. Instead the crab settled into a resting posture, and the antennule flicking and gill bailing gradually returned to their initial state.

Because the increase in antennule flicking and gill bailing always preceded any feeding or grooming behavior and occurred alone at low FDCE levels, it ap-

peared that the behavior of the antennules and gill bailers indicated detection of the FDCE and could be used to distinguish the levels of FDCE at which detection occurs from those levels at which food searching and gathering is released. To make this distinction the first experiment was performing using criteria for chemical detection and response with feeding movements, which were chosen from the behavioral sequences described above and which were defined in the Methods section.

*The threshold for detection of FDCE solutions*

The percentages of crabs detecting and responding with the feeding movement, chelae probing, were plotted against the logarithm of the estimated maximum FDCE concentration to which the crabs were exposed (Fig. 1). The FDCE concentrations at which 50% of the crabs detected the FDCE were calculated from the regression equations in Table I. Because the number of points where crabs exhibited feeding behavior was not adequate to construct a regression equation, the 50% threshold concentrations were estimated graphically (Fig. 1).

In the first experiment the feeding threshold was approximately 0.5 g/liter. In the second experiment crabs on *ad libitum* diet showed a feeding threshold of $10^{-2}$ g/liter and crabs deprived of food for six days had a lower feeding threshold of $10^{-3}$ g/liter.

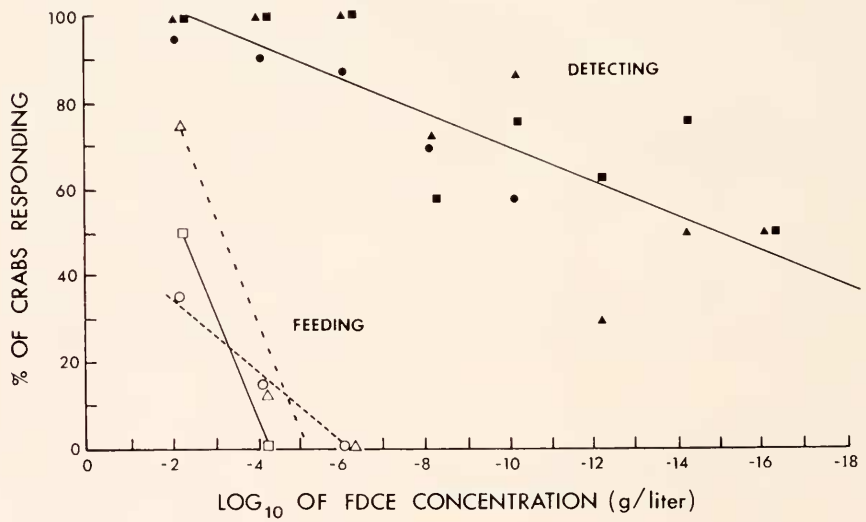


FIGURE 1. The percentage of blue crabs either detecting the FDCE or exhibiting feeding behaviors as a function of the logarithm of FDCE concentration: solid shapes indicate detecting; open shapes, feeding; circles represent the first experiment; squares, the second experiment with crabs on an *ad libitum* diet; triangles, the second experiment with crabs deprived of food for six days. In the first experiment each point represents 45 trials; in the second, 8 trials. For the first experiment the percentage of crabs detecting the control sea water was 34% (47 trials); for the second, 25% (8 trials) for crabs on an *ad libitum* diet and 38% (8 trials) for crabs after six days' food deprivation.

TABLE I

*Regression equations relating the percentage of crabs detecting the FDCE to the logarithm of the FDCE concentration: Y = percentage of crabs detecting; X = $\log_{10}$ [FDCE].*

| | Equation | $R^2$ | 95% Confidence intercept | Limits slope | [FDCE] at which 50% detection is predicted g/liter |
|---|---|---|---|---|---|
| First experiment | $Y = 109.78 + 4.94X$ | 0.960 | ±12.77 | ±1.89 | $7.9 \times 10^{-13}$ |
| Second experiment | | | | | |
| With food | $Y = 108.70 + 3.39X$ | 0.650 | ±25.42 | ±2.47 | $4.8 \times 10^{-18}$ |
| Without food | $Y = 116.99 + 4.76X$ | 0.707 | ±31.40 | ±3.06 | $8.5 \times 10^{-15}$ |
| Combining with and without food | $Y = 109.40 + 3.74X$ | 0.737 | ±22.96 | ±2.23 | $1.4 \times 10^{-16}$ |
| Combining all experiments | $Y = 109.50 + 3.92X$ | 0.664 | ±12.80 | ±1.34 | $6.5 \times 10^{-16}$ |

The detection thresholds in both experiments were several orders of magnitude lower than those for feeding. The regression equations for detection by crabs deprived of food and by crabs on an *ad libitum* diet did not differ significantly in slope or intercept and, therefore, were combined (Table I). The regression equation for detection in the second experiment also did not differ in slope or intercept from that of the first, but did show more variability. Because detection equations did not differ from experiment to experiment or with or without food, they were combined into one equation.

From this resultant equation the FDCE concentration at which 50% (±5.14 est. s.e.) of the crabs detect the FDCE proved to be $6.5 \times 10^{-16}$ g/liter. Back calculation of the 95% confidence limits about the regression line indicated that the detection threshold fell between $10^{-13}$ and $10^{-18}$ g/liter. The detection threshold was from $10^{10}$ to $10^{17}$ times lower than the feeding threshold and did not change after food deprivation for six days.

## DISCUSSION

With ablation experiments Hazlett (1971) has demonstrated that the antennules of *Callinectes sapidus* and most other decapods examined function as distance chemoreceptors. We have found that close attention to the antennular motion allows one to discern when a sapid substance is detected. The gill bailing accompanying the elevated antennule flicking rate increases the rate at which water flows out of the buccal area and around the antennules. The increased antennule flicking and gill bailing presumably magnifies the rate at which water is sampled and thus can be viewed as an active search by the crab for more information about its chemical environment.

In these experiments the linear regression line used to estimate thresholds is an approximation of the relationship between the percentage of crabs detecting the FDCE and the logarithm of its concentration. One can reasonably expect the extreme upper portion of the curve to become asymptotic to 100% because at very high FDCE concentration almost all the crabs will detect the FDCE. Similarly, because reactions of crabs to the control sea water indicate that a certain low percentage of the crabs will react to any change in the incoming water flow, it is

reasonable to expect the extreme lower portion of the curve to become asymptotic to some control value. Because the departure from linearity occurs at the extremes of the curve, the regression line can still be used to estimate the 50% threshold.

Fuzessery and Childress (1975) have discussed the postulate that among decapods contact chemoreception occurs at the less chemosensitive dactyls, while distance chemoreception occurs at the more sensitive antennules. The postulate suggests that the difference in feeding and detecting thresholds seen in *C. sapidus* may derive from a difference in chemosensitivity between dactyls and antennules. If the release of feeding behavior in *C. sapidus* requires stimulation of contact receptors on the dactyls or buccal region, this requirement may account for the high feeding threshold in *C. sapidus*.

The fact that low concentrations of sapid material release antennule flicking and gill bailing does not indicate, however, whether these behaviors constitute a reflex or involve integration at higher centers. The animal's history, both the evolutionary history of its species and the particular history of the individual under natural conditions, influences at what level of sapid solution the animal will show more complex behaviors such as food gathering. The question remains, therefore, whether the low levels of sapid material ($10^{-15}$ g/liter FDCE) provide sufficient information for the occurrence of complex behavior or whether such low levels only serve to prime or alert the animal.

Apparently, only Fuzessery and Childress (1975) have noted grooming as an alternative response to sapid solutions, as seen in *C. sapidus*. Because the blue crab rubs the palp over the antennule, chelae, dactyls, and mouthparts, which have high densities of chemoreceptors, one suspects that such grooming serves to clean the chemoreceptors. For the hermit crab, *Pagurus alaskensis*, Snow (1973) has found that antennular wiping by the endopodites removed debris from the aesthetasc hairs.

The startle response with subsequent defecation and vigorous grooming observed with high FDCE levels may be an avoidance reaction. In the spiny lobster, *Panulirus argus*, Levandowsky and Hodgson (1965) have observed avoidance responses to high levels of amino acids and amines that elicited feeding at lower levels. In this study, if such startle responses to high FDCE levels were equated to feeding responses, the estimated feeding threshold would drop in the first experiment to $10^{-2}$ g/liter, but would not change in the second experiment.

Comparison of thresholds for *Callinectes sapidus* with those of other crustaceans is difficult because the response criteria, sapid substances, and food deprivation schedules vary among investigations. Nevertheless, somewhat comparable results exist for five other crustaceans. For the lobster, *Homarus americanus*, McLeese (1974) has estimated the threshold concentration at which an extract of cod muscle released upstream walking to be $3 \times 10^{-5}$ g/liter. Using the regression equation of Mackie (1973) for the lobster, *H. gammarus*, the threshold at which a lipid-free squid mantle extract released gathering motions of the chelate pereiopods and upstream walking was calculated to be $2.1 \times 10^{-6}$ g/liter. Our regression of the data of Fuzessery and Childress (1975) has shown the threshold concentrations for the release of feeding motions by an equimolar mixture of three amino acids to be $6.8 \times 10^{-3}$ g/liter for *Pagurus hirsutiusculus*, $2.3 \times 10^{-7}$ g/liter

for *Pleuroncodes planipes,* and $3.0 \times 10^{-9}$ g/liter for *Cancer antennarius.* Because mixtures of amino acids alone have been found to be less attractive than food extracts or complex synthetic mixtures (Shelton and Mackie, 1971; Mackie, 1973), the last three crustaceans would perhaps have shown lower thresholds if they had been assayed with a food extract instead of an amino acid mixture. The major difference between this study of *C. sapidus* and those of the other crustaceans is that the response criteria for the other studies were limited to feeding motions, while for the blue crab an additional set of criteria was applied. When comparing the thresholds established with feeding motions, *C. sapidus* appears less sensitive to sapid materials than the other crustaceans except for *P. hirsutiusculus,* but the fact that the detection threshold of $10^{-15}$ g/liter for *C. sapidus* is lower than both its feeding threshold and those of the other crustaceans evinces that the behaviors indicating detection, *i.e.,* increased antennule flicking and gill bailing, are more sensitive response criteria than the feeding motions generally used. The blue crab's low detection threshold also suggests that crustaceans may detect lower levels of sapid substances than previously believed.

Mackie and Shelton (1972) found that, after nine days' starvation, the feeding threshold of *Homarus gammarus* decreased from $10^{-4}$ to $10^{-6}$ g/liter. After six days of food deprivation, the feeding threshold of *C. sapidus* dropped from $10^{-2}$ to $10^{-3}$ g/liter FDCE, but the detection thresholds did not change. Thus, food deprivation lowers the feeding threshold, but, at least for *C. sapidus,* does not bring the animal to respond at its limit of chemical detection or depress that limit. The remaining questions are whether food deprivation lowers the feeding threshold to some consistent degree and whether food deprivation longer than six days influences the detection threshold.

The increased antennule flicking rate and gill bailing may indicate not just the detection of sapid chemicals but the sensing of any chemical discontinuity in the crab's environment. If so, the behavioral assay based on the observation of antennular behavior could be used to investigate the detection of other chemicals important in the crab's life history, *e.g.,* those playing a part in habitat selection or social behavior. Also the reasonable assumption that the chemical milieu varies geographically leads us to expect that detection thresholds based upon sensing chemical discontinuities also vary geographically.

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## Summary

1. An increase in the rate of antennule flicking and gill bailing upon presentation of sea water solutions of a freeze-dried clam extract indicated detection of sapid substances by the blue crab, *Callinectes sapidus.*

2. The threshold concentration at which crabs detected the sapid solution was $10^{-15}$ g/liter. Feeding behaviors were released at higher concentrations, $10^{-1}$ to $10^{-2}$ g/liter.

3. Food deprivation for six days lowered the threshold for feeding behaviors but did not affect the detection threshold.

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